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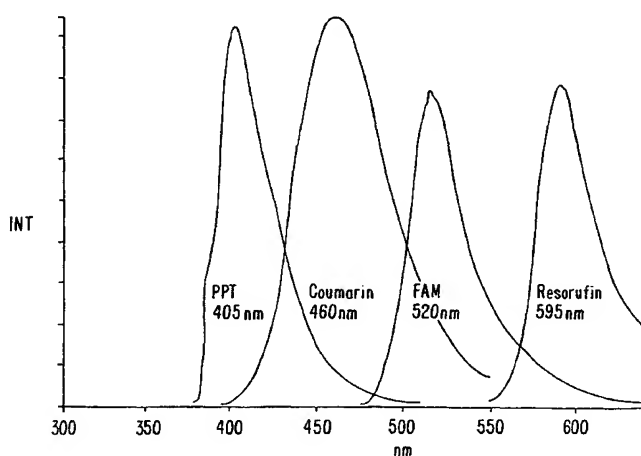
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(54) Title: FLUORESCENT QUENCHING DETECTION REAGENTS AND METHODS



(57) Abstract: Oligonucleotide-fluorophore-quencher conjugates wherein the fluorophore moiety has emission wavelengths in the range of about (300) to about (800) nm, and or where the quencher includes a substituted 4-(phenyldiazenyl)phenylamine structure provide improved signal to noise ratios and other advantageous characteristics in hybridization and related assays. The oligonucleotide-fluorophore-quencher conjugates can be synthesized by utilizing novel phosphoramidite reagents that incorporate the quencher moiety based on the substituted 4-(phenyldiazenyl)phenylamine structure, and or novel phosphoramidite reagents that incorporate a fluorophore moiety based on the substituted coumarin, substituted 7-hydroxy-3H-phenoxazin-3-one, or substituted 5,10-dihydro-10-[phenyl]pyrido[2,3-d:6,5-d']dipyrimidine-2,4,6,8-(1H, 3H, 7H, 9H, 10H)-tetrone structure. Oligonucleotide-fluorophore-quencher-minor groove binder conjugates including a pyrrolo[4,5-e]indolin-7-yl carbonylpyrrolo[4,5-e]indolin-7-yl carbonylpyrrolo[4,5-e]indoline-7-carboxylate (DPI₃) moiety as the minor groove binder and the substituted 4-(phenyldiazenyl)phenylamine moiety as the quencher, were synthesized and have substantially improved hybridization and signal to noise ratio properties.



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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No

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A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07H21/00 C07C245/08 C07F9/24 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07H C07C C07F C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| X | ASANUMA H ET AL: "Photo-Responsive Oligonucleotides Carrying Azobenzene in the Side-Chains" TETRAHEDRON LETTERS,NL,ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, vol. 39, no. 49, 3 December 1998 (1998-12-03), pages 9015-9018, XP004140991 ISSN: 0040-4039 scheme 1 | 6 |



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

° Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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- * & * document member of the same patent family

Date of the actual completion of the international search

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Date of mailing of the international search report

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Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

International Application No

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| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
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| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| A | LEE L G ET AL: "ALLELIC DISCRIMINATION BZ NICK-TRANSLATION PCR WITH FLUOROGENIC PROBES" NUCLEIC ACIDS RESEARCH,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 21, no. 16, 11 August 1993 (1993-08-11), pages 3761-3766, XP000470188 ISSN: 0305-1048 cited in the application page 3761 -page 3762 --- | 1,57 |
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INTERNATIONAL SEARCH REPORT

Int'l. Application No

PCT/US 00/33333

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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| A | US 5 801 155 A (GAMPER HOWARD B ET AL) 1 September 1998 (1998-09-01) claims ----- | 15,16 |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/33333

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-21, 22-28 (in part), 29 (in full),
30-33 (in part), 41-42 (in full), 57-58 (in full),
59-65 (in part), 66-67 (in full), 68-74 (in part),
75 (in full), 76-78 (in part)

A quencher moiety containing derivative where the quencher has the structure of the quencher moiety of claim 1.

2. Claims: 22-28 (in part), 30-33 (in part), 34-40 (in full),
43-56 (in full), 59-65 (in part), 68-74 (in part),
76-78 (in part)

A fluorophore containing derivative where the fluorophore has one of the structures of claim 22 and which is not falling within one of the previously described subjects.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/33333

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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